CANADIAN JOURNAL OF RESEARCH

VOLUME 20 MARCH, 1942 NUMBER 3

CONTENTS

SECTION A.—PHYSICAL SCIENCES

Page

Absorption of Light and Heat Radiation by Small Spherical Particles. II. Scattering of Light by Small Carbon Spheres —*R. Ruedy* - - - - - - - - - - - - - - 25

SECTION B.—CHEMICAL SCIENCES

Contribution à l'Étude des Semicarbazides δ-Substituées. III. Essai d'Application au Dosage du Glucose—*G. H. Baril, R. Barré, and L. Piché* - - - - - - - - - - - 33

Effect of Initial Drying Temperature on the Apparent Lignin Content of Plant Tissues—*D. MacDougall and W. A. DeLong* 40

The Alkaloids of Fumariaceous Plants. XXXI. *Corydalis montana* (Engelm.) Britton.—*R. H. F. Manske* - - - 49

NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

Publications and Subscriptions

The Canadian Journal of Research is issued monthly in four sections, as follows:

A. Physical Sciences
B. Chemical Sciences
C. Botanical Sciences
D. Zoological Sciences

For the present, Sections A and B are issued under a single cover, as also are Sections C and D, with separate pagination of the four sections, to permit separate binding, if desired.

Subscription rates, postage paid to any part of the world (effective 1 April, 1939), are as follows:

	Annual	*Single Copy*
A and B	$ 2.50	$ 0.50
C and D	2.50	0.50
Four sections, complete	4.00	—

The Canadian Journal of Research is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. All correspondence should be addressed:

National Research Council, Ottawa, Canada.

Notice to Contributors

Fifty reprints of each paper are supplied free. Additional reprints, if required, will be supplied according to a prescribed schedule of charges.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. A. MARCH, 1942 NUMBER 3

ABSORPTION OF LIGHT AND HEAT RADIATION BY SMALL SPHERICAL PARTICLES

II. SCATTERING OF LIGHT BY SMALL CARBON SPHERES[1]

BY R. RUEDY[2]

Abstract

Spheres of carbon for which $2a/\lambda$, the ratio between the diameter of the particle and the incident wave-length, is less than about $\frac{1}{10}$ scatter the light uniformly in all directions. The intensity of the scattered radiation for any angle is proportional to the square of the volume of the particle and inversely proportional to the fourth power of the wave-length. As the ratio $2a/\lambda$ increases from $\frac{1}{4}$ to $\frac{1}{2}$ and greater values, the diffused light collects more and more into a main beam that appears as a continuation of the incident ray and that decreases in width as $2a/\lambda$ increases. Blue light prevails in the scattered radiation. When the size of the particles is unknown, the intensity, distribution, and polarization of the scattered light give an at least approximate value for the radius.

Amount of Light Scattered by Small Spherical Particles of Carbon

The loss of light observed behind a small spherical obstacle brought into the path of a plane wave of light may be large for two reasons, either because a large portion of the incident light that enters the obstacle is transformed into heat, or because a large portion of the incident light is deflected in other directions by the obstacle. It is to be expected that if the sphere consists of carbon, absorption accounts for the larger part of the loss; indeed, the relatively high index of refraction of carbon indicates that the electrons yield readily to an external alternating field; the conductivity, however, is moderate and the ohmic losses caused by the motion of the electrons are considerable.

In order to determine exactly what fraction of the total loss measured behind a spherical carbon particle is due merely to scattering or diffraction of the light, and what fraction is lost by true absorption, it is necessary to consider both the real and the imaginary part of the amplitudes a_ν and p_ν of the component electric and magnetic waves for which the solution of the problem is obtained. For an incident wave of unit intensity in air these amplitudes are as follows.

[1] *Manuscript received November 22, 1941.*

Contribution from the Research Plans and Publications Section, National Research Laboratories, Ottawa, Canada. Issued as N.R.C. No. 1032.

Part I, containing the general formulae, appeared in Can. J. Research, 19 (10) : 117-125. 1941.

[2] *Research Investigator.*

$$a_\nu = \frac{(-1)^\nu(2\nu+1)}{-i+(-1)^\nu \dfrac{J_{-\nu-\frac{1}{2}}(\alpha)}{J_{\nu+\frac{1}{2}}(\alpha)} \dfrac{\frac{\beta}{\alpha}\dfrac{J'_{-\nu-\frac{1}{2}}(\alpha)}{J_{-\nu-\frac{1}{2}}(\alpha)} - \dfrac{J'_{\nu+\frac{1}{2}}(\beta)}{J_{\nu+\frac{1}{2}}(\beta)}}{\frac{\beta}{\alpha}\dfrac{J'_{\nu+\frac{1}{2}}(\alpha)}{J_{\nu+\frac{1}{2}}(\alpha)} - \dfrac{J'_{\nu+\frac{1}{2}}(\beta)}{J_{\nu+\frac{1}{2}}(\beta)}}}$$

$$p_\nu = \frac{(-1)^{\nu+1}(2\nu+1)}{-i+(-1)^\nu \dfrac{J_{-\nu-\frac{1}{2}}(\alpha)}{J_{\nu+\frac{1}{2}}(\alpha)} \dfrac{\frac{\beta}{\alpha}\dfrac{J'_{\nu+\frac{1}{2}}(\beta)}{J_{\nu+\frac{1}{2}}(\beta)} - \dfrac{J'_{-\nu-\frac{1}{2}}(\alpha)}{J_{-\nu-\frac{1}{2}}(\alpha)}}{\frac{\beta}{\alpha}\dfrac{J'_{\nu+\frac{1}{2}}(\beta)}{J_{\nu+\frac{1}{2}}(\beta)} - \dfrac{J'_{\nu+\frac{1}{2}}(\alpha)}{J_{\nu+\frac{1}{2}}(\alpha)}}}.$$

In the present instance, when $\alpha = 2\pi a/\lambda$ the derivatives

$$J'_{\nu+\frac{1}{2}}(\alpha) = J_{\nu-\frac{1}{2}}(\alpha) - \frac{\nu}{\alpha} J_{\nu+\frac{1}{2}}(\alpha)$$

$$J'_{-\nu-\frac{1}{2}}(\alpha) = -J_{-\nu+\frac{1}{2}}(\alpha) - \frac{\nu}{\alpha} J_{-\nu-\frac{1}{2}}(\alpha),$$

are approximately equal to the derivatives of the Bessel functions of half-integral order. The real part and the imaginary part of each of the components a_ν and p_ν are entirely different functions of the ratio $2a/\lambda$ or of $\alpha = 2\pi a/\lambda$, as a glance at Figs. 1 to 4 will show. For very small values of α the value of a_ν increases as $\alpha^{2\nu+1}$; that for p_ν increases as $\alpha^{2\nu+3}$. For larger and larger values of α, the rate of increase of a_ν and p_ν diminishes, and after a few fluctuations, a_ν and p_ν vary regularly according to the law

$$a_\nu = \frac{2\nu+1}{n-in\kappa+1} i^\nu(\cos\alpha + i\sin\alpha)\left((n-in\kappa)i\cos\left(\alpha-\nu\frac{\pi}{2}\right)+\sin\left(\alpha-\frac{\nu\pi}{2}\right)\right)$$

$$-p_\nu = \frac{2\nu+1}{n+1-in\kappa} i^\nu(\cos\alpha+i\sin\alpha)\left(i\cos\left(\alpha-\nu\frac{\pi}{2}\right)+(n-in\kappa)\sin\left(\alpha-\frac{\nu\pi}{2}\right)\right).$$

It follows that in this final stage the real parts of a_ν and p_ν oscillate between

$$\pm\frac{2\nu+1}{2}\frac{1-n^2-n^2\kappa^2}{(1+n)^2+n^2\kappa^2},$$

the positive values occurring for multiples of π, the negative for odd multiples of $\pi/2$ when the formula is used for a_ν (Figs. 2 and 4). For surroundings other than air, n has to be divided by the refractive index m_0 of the medium.

The imaginary portions of a_ν and p_ν, on the other hand, reach much higher values (Figs. 1 and 3); apart from the sign, which remains unchanged, these values alternate regularly between

$$(2\nu+1)\frac{1+n}{(1+n)^2+n^2\kappa^2} \text{ and } (2\nu+1)\frac{n^2+n+n^2\kappa^2}{(1+n)^2+n^2\kappa^2},$$

and are thus simply proportional to $(2\nu+1)$.

When, therefore, larger and larger ratios are chosen for α, the imaginary part of the difference $(-1)^\nu(a_\nu - p_\nu)$, which determines the total loss of light along the direction of the incident ray, becomes equal to $i\nu^2$. Calculations show the number ν of components that contribute appreciably to

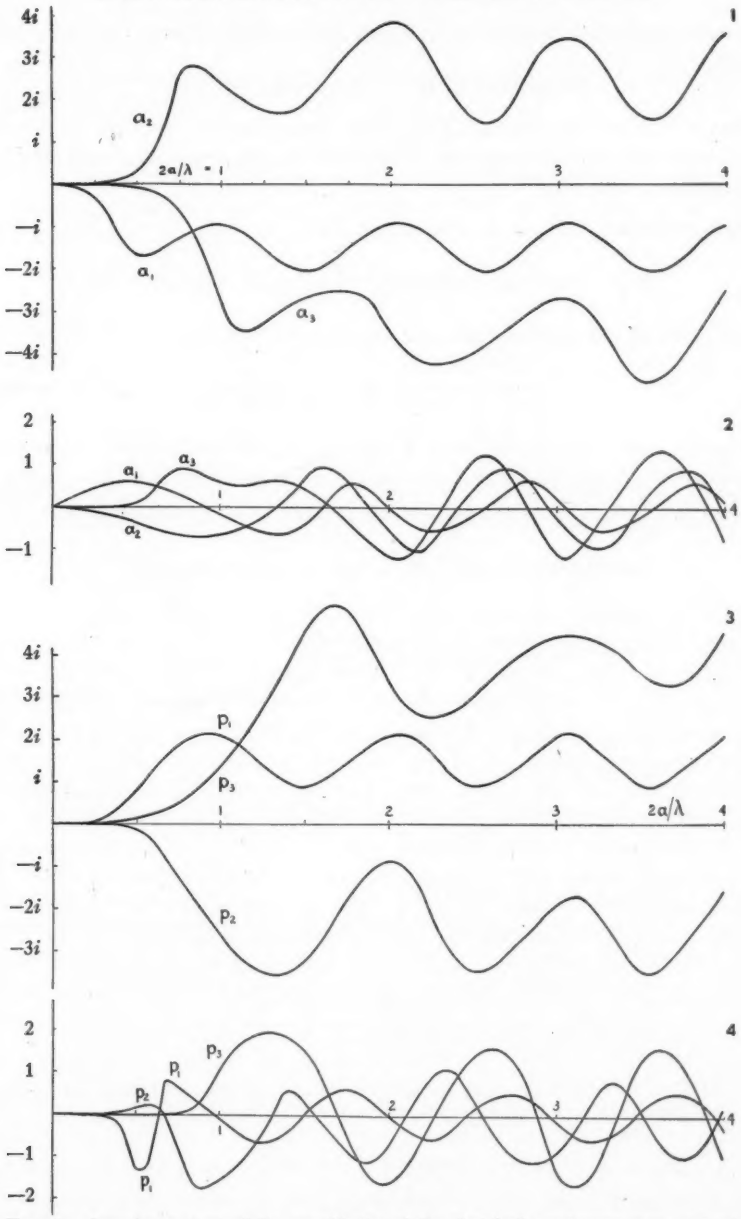

FIG. 1. *Imaginary part of the amplitudes of the electrical component waves set up by spherical carbon particles of different ratios* $2a/\lambda$. FIG. 2. *Real part of the amplitudes of the electrical component waves set up by the particles.* FIG. 3. *Imaginary part of the amplitudes of the magnetic component waves sent out by particles having different ratios* $2a/\lambda$. FIG. 4. *Real part of the amplitudes of the component magnetic waves sent out by carbon particles.*

the sum of all the expressions $(-1)^{\nu}(a_{\nu} - p_{\nu})$ to be equal to α; there follows the relation

$$\text{imag. part of } \Sigma(-1)^{\nu}(a_{\nu} - p_{\nu}) = \alpha^2 .$$

Hence the actual surface, $2\pi a^2$, that the obstacle presents to the light, and not merely its cross-section, is involved in the absorption and deflection of the wave.

Of the fraction lost per unit length of path,

$$k = \text{imaginary component of } \frac{\lambda^2}{2\pi m_0^2}\Sigma(-1)^{\nu}(a_{\nu} - p_{\nu}),$$

when there is one particle per unit volume, the amount

$$k_s = \frac{\lambda^2}{2\pi m_0^2}\,\Sigma\,\frac{|a_{\nu}|^2 + |p_{\nu}|^2}{2\nu + 1}$$

is lost as a result of the deflection of the light in other directions. The symbol $|a|$ means the absolute value of the complex quantity a. The values calculated for carbon particles are shown in Fig. 5 for various diameters of the

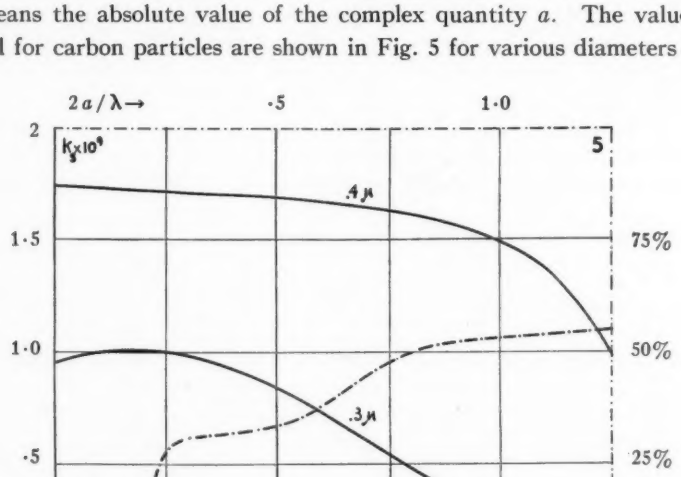

FIG. 5. *Loss of light caused by scattering for one particle per unit volume and percentage of total loss caused by diffusion (dot-dash lines).*

particle ($2a = 0.15, 0.2, 0.3$, and $0.4\ \mu$). They are seen to be small in the visible region at least, and to decrease in general with the wave-length, most steeply when the diameter $2a$ of the particle is in the neighbourhood of 0.3μ; for this size the loss in the visible diminishes markedly as the wave-length is increased.

Ratio Between Loss by Deflection and Total Loss

For very small values of α, only one component, the electric wave of amplitude a_1, where

$$a_1 = 2\alpha^3 \frac{(n - in\kappa)^2 - 1}{(n - in\kappa)^2 + 2},$$

need be considered. It follows that the ratio of deflected light to the total fraction of the radiation lost along the direction of the incoming wave, owing to the presence of the particle, is equal to

$$R = 2\alpha^3 \frac{(n^2 - n^2\kappa^2 - 1)(n^2 - n^2\kappa^2 + 2) - i6n\kappa}{(n^2 - n^2\kappa^2 + 2)^2 + 4n^2\kappa^2}.$$

The ratio is zero when the particles are extremely small but increases considerably as values between $\pi/10$ and $\pi/4$ are given to α. For very large values of α the fraction tends towards the limit

$$R_l = \frac{(n^2 + n + n^2\kappa^2)^2 + (1 + n)^2 + 2n^2\kappa^2}{((1 + n)^2 + n^2\kappa^2)^2},$$

or towards about 0.6 for carbon particles. Fig. 5 shows the changes in the proportion of diffracted or scattered light for carbon particles of various sizes.

Angular Distribution of the Deflected Light

The amount of light that is deflected by a spherical obstacle depends on the angle between the direction considered and the direction of the incident wave. The variation of the intensity with the angle merits examination on account of its close relation to the size of the particle.

When a spherical particle is placed in the path of a plane unpolarized wave of strong monochromatic light, the intensity of the scattered light observed in a direction that forms the arbitrary angle γ with the incident beam sent along the horizontal or z-axis is obtained by the simple addition of the two polarized components $I_\perp$ and $I_\parallel$. The intensity $I_\perp$ is the intensity of the light whose electrical vibration is perpendicular to the plane containing the line of vision and the incident ray, and $I_\parallel$ is the intensity of the light whose electric vector oscillates in the plane determined by the line of vision and the incident ray. When the distance r separating observer and particle is a large multiple of a, the general formulae for the intensity $I_\perp$ and $I_\parallel$ are

$$I_\perp = \frac{1}{\rho^2}\left|\sum_1^\infty \left(\frac{a_\nu}{\nu(\nu+1)}\,\Pi_\nu + \frac{p_\nu}{\nu(\nu+1)}\,(v\Pi_\nu - (1 - v^2)\Pi'_\nu)\right)\right|^2$$

$$I_\parallel = \frac{1}{\rho^2}\left|\sum_1^\infty \left(\frac{a_\nu}{\nu(\nu+1)}\,(v\Pi_\nu - (1 - v^2)\Pi'_\nu) + \frac{p_\nu}{\nu(\nu+1)}\,\Pi_\nu\right)\right|^2,$$

where

$$v = \cos\gamma$$

$$\rho = \frac{2\pi r}{\lambda} m_0$$

$$\Pi_\nu = \frac{1}{2^\nu} \sum_0^{\frac{\nu+1}{2}-1} (-1)^s \frac{(2\nu - 2s)!\, v^{\nu-2s-1}}{(\nu - s)!\, s!(\nu - 2s - 1)!}.$$

When the upper limit of $s = (\nu + 1)/2$ is half an integer, this particular value must not be considered in evaluating Π_ν.

The sums $(I_\perp + I_\parallel)$ to be expected at a given large distance from carbon particles of different diameters have been calculated, and are represented in Figs. 6 to 9 along the directions for which they are valid; the small spheres are assumed to be of such size that when illuminated with light of a constant wave-length the ratio $2a/\lambda$ is a multiple of $\frac{1}{8}$. Grains for which $2a/\lambda$ is less than $\frac{1}{8}$ are seen to diffuse light uniformly in all directions. For ratios from $\frac{1}{4}$ upwards, light is deflected only slightly from the direction of the incoming ray. For $2a/\lambda = \frac{1}{2}$, for instance, by far the larger part of the light coming from the particle is confined to the space within the angles 120° and 240°. With still larger spheres (Fig. 9) only a narrow main beam, accompanied by a few unimportant side lobes, remains and very little light is visible at right angles to the direction of the incoming wave.

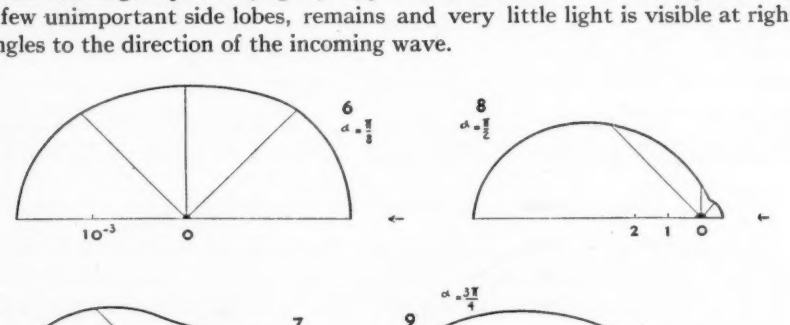

FIG. 6. *Distribution of scattered light of a given wave-length around a spherical carbon particle for which* $2a/\lambda = \frac{1}{8}$. *The light is coming from the right.*

FIG. 7. *Distribution of scattered light around a spherical carbon particle for which* $2a/\lambda = \frac{1}{4}$.

FIG. 8. *Distribution of scattered light around a spherical carbon particle for which* $2a/\lambda = \frac{1}{2}$.

FIG. 9. *Distribution of scattered light around a spherical carbon particle for which* $2a/\lambda = \frac{3}{4}$. *Scale units are 10,000 times as large as those used for* $2a/\lambda = \frac{1}{8}$ *and 100 times as large as those used for* $2a/\lambda = \frac{1}{4}$.

As regards the intensity of the radiation at a given distance, only a small amount of light is diffused by particles having a diameter less than one-eighth of the wave-length of the incident light. For $2a/\lambda = \frac{1}{8}$, the light contributed by 10,000 particles is required to equal the intensity obtained in any direction from a sphere for which $2a/\lambda = \frac{3}{4}$. According to the formula valid for the smallest particles,

$$I_\perp = \frac{\lambda^2}{16\pi^2 r^2} |a_1|^2$$

in any direction. When the volume $V = 4\pi a^3/3$ is introduced, this expression can be written

$$I_\perp = \frac{9V^2}{4r^2\lambda^4} \frac{|a_1|^2}{\alpha^6} = \frac{9V^2}{r^2\lambda^4} \left| \frac{(n - in\kappa)^2 - 1}{(n - in\kappa)^2 + 2} \right|^2,$$

whereas I_{II} is negligible. That is to say, the intensity of the scattered light in any direction at a distance r from a carbon sphere for which $2a/\lambda$ is less than about $\frac{1}{10}$ is proportional to the sixth power of the radius a and inversely proportional to the fourth power of the wave-length. A steep increase in the intensity of scattered light occurs therefore with increase in diameter. But for larger and larger obstacles, the outgoing light becomes concentrated along the direction of the incoming ray; the intensity of this light varies more nearly as the volume or mass of the particle of carbon, or even less steeply. When, for instance, the intensities obtained behind a spherical obstacle for which $2a/\lambda = 0.5$ are multiplied by $3\frac{3}{8}$ (the ratio between the volumes or masses of spheres for which $2a/\lambda = 0.5$, in one case, and $2a/\lambda = 0.75$ in the other), the results obtained exceed the values given by the larger particles, or are at least of the same order of magnitude (Figs. 8 and 9).

Polarization of Scattered Light

In the light deflected and scattered by the particle along a given horizontal direction, the intensity furnished by the vibration that occurs parallel to the vertical or x-axis is no longer equal to the intensity of the vibration in the horizontal plane. There is therefore in the scattered light an excess $I_I - I_{II}$ or a fraction $(I_I - I_{II})/(I_I + I_{II})$ of polarized light. The vibrations are parallel to the vertical axis when the fraction is positive, and parallel to the plane passing through the incident ray and the direction of vision when it is negative. The fraction varies with the angle γ and the size of the particles. For light scattered by molecules, the radiation emitted in a direction that forms 90° with the incoming ray is completely polarized, the electrical vibrations occurring in the direction that is normal to the plane passing through the incoming and the outgoing rays. The same result is obtained with small spherical carbon particles, as long as the ratio $2a/\lambda$ is less than about $\frac{1}{4}$. When this fraction exceeds $\frac{1}{4}$, the polarization is never quite complete whatever the direction of vision, but the percentage of polarized light attains a maximum for a certain angle or for several angles. For $2a/\lambda = \frac{1}{2}$, the maximum is equal to about $\frac{3}{8}$ near 90°; for $2a/\lambda = \frac{3}{4}$ there occurs almost complete polarization for an angle of 60°, where however the intensity of scattered light is very weak; for directions between 80° and 110° the electrical vibrations in the very faint scattered radiation are parallel to the plane containing the incoming ray and the direction of vision. At 120° the extent of polarization passes through a second peak of about 50%. This variation is a useful indication of the size of the particles.

Practical Applications

The light transmitted or scattered by a single grain of carbon is too weak to be studied; but if a small cloud of particles of similar sizes is contained within a small space, the angles between the incoming and outgoing rays remain virtually unchanged for a distant observer, whatever the particle

chosen, and the formulae remain valid for the total radiation received from the accumulation of N particles.

The most straightforward application of the theory consists of the calculation of the optical properties that a known number of carbon particles of diameter $2a$ suspended in unit volume of air would possess either in white or in monochromatic light.

In practice the reverse problem may also occur; when absorption and scattering per unit volume have been determined experimentally, the concentration of the particles and their size, if approximately uniform, have to be found. If it is possible to measure the relative amount of scattered light at a few angles and for one state of polarization, and if the intensities obtained are plotted as a function of the angles, a comparison of the curve with the theoretical curves for different values of $2a/\lambda$ (Figs. 6 to 9, for instance) suggests an approximate value for the diameter of the particle. A uniform distribution of the diffused light is proof of a diameter less than about $\lambda/10$; absence or weakness of scattered light along directions that form acute angles with the direction of the incident wave points to the presence of coarse carbon sphericles for which $2a/\lambda$ is greater than $\frac{1}{2}$.

As soon as the size is known, the coefficient of absorption can be computed for one particle in unit volume, and the number of particles N can be deduced from the measured absorption per unit volume of suspension. The result arrived at for the concentration of the spheres can be verified by measuring the actual intensity of the diffused light, expressed as a fraction of the incident light, along at least one direction of observation, and by comparing it with the calculated intensity for N grains of carbon.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. B. MARCH, 1942 NUMBER 3

CONTRIBUTION À L'ÉTUDE DES SEMICARBAZIDES δ-SUBSTITUÉES

III. ESSAI D'APPLICATION AU DOSAGE DU GLUCOSE[1]

PAR GEORGES H. BARIL[2], ROGER BARRÉ[3] ET LUCIEN PICHÉ[4]

Sommaire

Nous avons tenté d'adapter la *p*-nitrophényl-4-semicarbazide à une méthode de dosage gravimétrique du glucose.

La réaction de ce dérivé sur le dextrose fournit une molécule d'eau comme produit secondaire; afin de supprimer l'équilibre qui tend à limiter le rendement, cette eau est éliminée par évaporation au fur et à mesure de sa formation. On obtient ainsi en peu de temps un rendement quantitatif de glucose-*p*-nitrophényl-4-semicarbazone qu'on dose par précipitation à l'état de *m*-nitrobenzal-semicarbazone insoluble.

Cette méthode permet, sans défécation, de doser le glucose dans les milieux réducteurs; elle est applicable directement à des concentrations de glucose comprises entre 0.2 à 1.0% et peut atteindre une concentration limite de 0.1% par concentration préalable de la solution sucrée.

Introduction

Dans une communication précédente (2), nous avons signalé que les semicarbazides δ-substituées fournissent rapidement un rendement élevé, sinon théorique, de semicarbazones peu solubles lorsqu'on les fait réagir avec les aldéhydes ou les cétones. C'est en nous basant sur cette propriété que nous avons essayé de mettre au point une méthode de dosage gravimétrique du glucose. Nous nous sommes adressés à la *p*-nitrophényl-4-semicarbazide comme réactif parce qu'elle présente l'affinité et la stabilité nécessaires à cette fin.

Le procédé que nous proposons n'a pas la prétention d'être définitif; il se propose bien plus de démontrer la possibilité de baser un dosage du glucose sur une réaction spécifique, correspondant rigoureusement à une équation chimique et de la dégager ainsi des conditions aléatoires qui accompagnent la défécation des solutions et les méthodes à réduction d'un hydroxyde métallique en milieu alcalin.

[1] *Manuscrit original reçu le 9 octobre 1941, et sous forme revisée le 12 janvier 1942. Contribution de l'Institut de Chimie de la Faculté des Sciences, Université de Montréal, Montréal, Qué.*

[2] *Directeur de l'Institut de Chimie.*

[3] *Professeur titulaire de Chimie organique à la Faculté des Sciences.*

[4] *Chargé de cours en Chimie générale à la Faculté des Sciences.*

Partie théorique

L'insolubilisation progressive des semicarbazones du glucose que nous avons déjà indiquée (2) aurait constitué la clef de la méthode que nous nous proposons d'élaborer, sans une insolubilisation à peu près proportionnelle des semicarbazides elles-mêmes. En effet, il est nécessaire pour permettre aux semicarbazides substituées de se dissoudre en présence de la solution aqueuse du sucre, d'additionner celle-ci d'alcool ou d'un solvant approprié. Cette addition a pour effet de retenir en même temps une quantité appréciable de semicarbazone; lorsque celle-ci se forme et demeure en solution, un équilibre chimique s'établit entre la semicarbazide et la semicarbazone et il empêche la réaction d'être totale. Il n'est pas possible non plus, dans ces conditions, d'évaluer directement la quantité de glucose-semicarbazone qui s'est formée parce qu'elle ne précipite pas intégralement.

On peut cependant modifier les conditions de la réaction de façon à obtenir quand même un rendement quantitatif de glucose-semicarbazone; on peut théoriquement y arriver si on élimine l'eau produite au cours de la réaction, afin de supprimer l'équilibre. Il importe toutefois de noter que le glucose à doser se trouve inévitablement en solution aqueuse; l'eau de cette solution devra donc être éliminée en même temps que celle qui est fournie par la réaction.

Le problème de l'élimination de l'eau des systèmes en équilibre présente plusieurs solutions; on peut, par exemple, lui substituer un solvant moins volatil et soumettre le mélange réactionnel à la distillation ou à une simple évaporation. Des essais préliminaires au moyen de la *p*-nitrophényl-4-semicarbazide nous avaient en effet montré que l'addition de quantités croissantes d'alcool absolu à une solution aqueuse de glucose fournit des rendements de glucose-semicarbazone qui se rapprochent de plus en plus du rendement théorique. Le diagramme suivant correspond à cinq condensations pour lesquelles la concentration de l'eau a varié de 6 à 75%, du fait de l'addition

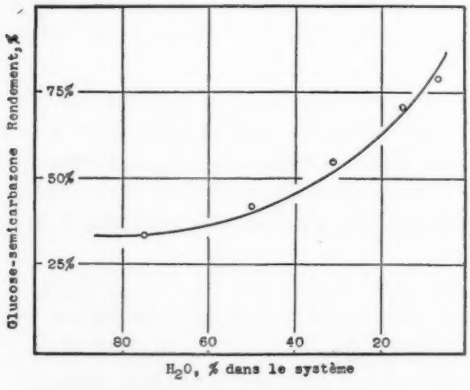

FIG. 1.

d'alcool; le rendement de glucose-semicarbazone a par le fait même augmenté de 33 à 78% du rendement théorique. L'allure de la courbe confirme l'hypothèse théorique, c'est-à-dire que l'élimination complète de l'eau du système, au fur et à mesure de sa formation, conduira éventuellement à un rendement quantitatif de glucose-semicarbazone:

$$CH_2OH—(CHOH)_4—CHO + \underset{\alpha}{NH_2}—\underset{\beta}{NH}—\underset{\gamma}{CO}—\underset{\delta}{NH}—C_6H_4NO_2 \longrightarrow$$

$$\longrightarrow H_2O + CH_2OH—(CHOH)_4—CH=\underset{\alpha}{N}—\underset{\beta}{NH}—\underset{\gamma}{CO}—\underset{\delta}{NH}—C_6H_4NO_2$$

Pour l'élimination de l'eau du système en équilibre, nous avons substitué l'alcool amylique à l'éthanol; il bout à 132° C. et permet ainsi une élimination rapide de l'eau par évaporation; d'autre part, il dissout bien la *p*-nitrophényl-4-semicarbazide et sa glucose-semicarbazone.

Dans ces nouvelles conditions, la glucose-semicarbazone ne peut plus précipiter, et il nous faut disposer d'une méthode qui permette de la doser facilement, en présence de la semicarbazide libre qui l'accompagne inévitablement. Cette méthode consiste à ajouter au mélange un léger excès de *m*-nitrobenzaldéhyde; ce réactif précipite quantitativement à froid la *p*-nitrophényl-4-semicarbazide sans affecter la glucose-semicarbazone correspondante. En outre, la *m*-nitrobenzal-semicarbazone qui se forme est très peu soluble même dans les milieux alcooliques additionnés d'alcool amylique.

Il suffit donc de filtrer la solution qui a été additionnée de *m*-nitrobenzaldéhyde pour la débarrasser de la *p*-nitrophényl-4-semicarbazide libre qu'elle contient. En chauffant maintenant le filtrat après l'avoir acidifié légèrement, on obtient l'hydrolyse de la glucose-semicarbazone; cette hydrolyse libère de la *p*-nitrophényl-4-semicarbazide qui se combine aussitôt à son tour à la *m*-nitrobenzaldéhyde pour donner un nouveau précipité de *m*-nitrobenzal-*p*-nitrophényl-4-semicarbazone insoluble; le poids de cette semicarbazone correspond à celui de la glucose-semicarbazone et, par extension à celui du glucose lui-même:—

$$CH_2OH—(CHOH)_4—CH=\underset{\alpha}{N}—\underset{\beta}{NH}—\underset{\gamma}{CO}—\underset{\delta}{NH}—C_6H_4NO_2 + H_2O \longrightarrow$$

$$\longrightarrow CH_2OH—(CHOH)_4—CHO + \underset{\alpha}{NH_2}—\underset{\beta}{NH}—\underset{\gamma}{CO}—\underset{\delta}{NH}—C_6H_4NO_2$$

$$NO_2C_6H_4—CHO + \underset{\alpha}{NH_2}—\underset{\beta}{NH}—\underset{\gamma}{CO}—\underset{\delta}{NH}—C_6H_4NO_2 \longrightarrow$$

$$\longrightarrow NO_2C_6H_4CH=\underset{\alpha}{N}—\underset{\beta}{NH}—\underset{\gamma}{CO}—\underset{\delta}{NH}—C_6H_4NO_2 + H_2O$$

Les rapports arithmétiques entre les poids moléculaires des substances qui figurent dans ces deux équations indiquent que 180.1 g. de glucose correspondent à la formation de 358.2 g. de glucose-semicarbazone puis à la précipitation de 329.2 g. de semicarbazone de la *m*-nitrobenzaldéhyde. Le poids de glucose qui est passé à l'état de semicarbazone au cours d'une condensation peut dès lors être calculé en multipliant par 0.5471 (180.1/329.2) le poids de *m*-nitrobenzal-semicarbazone que l'on recueille.

Méthode et résultats

Pour le dosage expérimental du glucose par la *p*-nitrophényl-4-semicarbazide, nous avons adopté la méthode suivante qui est basée sur les données théoriques précédentes: à 10 cm.^{3} de solution de glucose, contenant de 0.02 à 0.1 g. de ce sucre, on ajoute une quantité de *p*-nitrophényl-4-semicarbazide qui est à peu près proportionnelle à la quantité de glucose, déterminée approximativement par un essai préliminaire (0.1 g. de semicarbazide si la quantité de glucose est voisine de 0.02 ou 0.03 g.; 0.2 g. de semicarbazide si le glucose atteint 0.05 g. environ; ou 0.3 g. de semicarbazide si l'on a jusqu'à 0.1 g. de sucre). On y mélange 20 cm.^{3} d'alcool amylique et 1 cm.^{3} de solution à 10% d'acide orthophosphorique puis on porte au bain-marie bouillant. L'emploi d'acide phosphorique est justifié par les travaux d'Ardagh et Rutherford (1), qui ont démontré la propriété catalytique supérieure de l'acide phosphorique pour l'obtention des hydrazones et des combinaisons analogues.

Pour faciliter l'élimination rapide de l'eau et assurer en même temps l'homogénéité du milieu, on ajoute au mélange 40 cm.^{3} d'alcool éthylique absolu. Lorsque le volume est réduit par évaporation à 12 ou 15 cm.^{3}, ce qui prend environ 45 min., l'eau est depuis quelque temps déjà totalement éliminée et la condensation du glucose est complète. On retire du bain, on ajoute 5 cm.^{3} d'alcool absolu et on refroidit rapidement jusqu'à 22° à 25° C.

On procède alors à l'élimination de l'excès de *p*-nitrophényl-4-semicarbazide en ajoutant 5 cm.^{3} d'eau et 5 cm.^{3} de solution alcoolique à 10% de *m*-nitrobenzaldéhyde; la précipitation de la semicarbazone s'amorce immédiatement et peut être considérée complète au bout d'une heure et quarante minutes à la température de 22° à 25° C. Après filtration, la solution ne contient plus que la glucose-*p*-nitrophényl-4-semicarbazone en présence d'un excès de *m*-nitrobenzaldéhyde; on dose le glucose combiné en ajoutant à la solution, quatre gouttes d'acide chlorhydrique concentré et en la portant sur bain-marie pendant encore 30 min. Il se produit rapidement un deuxième précipité de *m*-nitrobenzal-*p*-nitrophényl-4-semicarbazone, en belles aiguilles soyeuses; on recueille sur entonnoir taré et on lave à l'eau puis on sèche à l'étuve pendant une heure à 105° à 110° (0.20 g. de *m*-nitrobenzal-semicarbazone laissés 48 h. à cette température n'ont accusé aucun changement de poids).

Le poids du précipité recueilli multiplié par le facteur 0.5471 correspond au poids de glucose présent dans la solution originale.

Cette méthode, appliquée d'abord à des solutions pures de glucose (Dextrose anhydre, échantillon No 41 du Bureau des Standards, Washington) a donné les résultats présentés au Tableau I.

Le protocole qui vient d'être exposé peut être modifié pour permettre d'atteindre des concentrations en glucose inférieures à celles qui figurent au Tableau I. Lorsque la concentration en glucose est inférieure à 0.20%, on peut prendre 50 cm.^{3} de solution sucrée, y ajouter 100 cm.^{3} d'alcool absolu et les autres solvants ou réactifs, dans les proportions qui ont déjà été indiquées;

TABLEAU I

DOSAGE EXPÉRIMENTAL DU GLUCOSE PAR LA *p*-NITROPHÉNYL-4-SEMICARBAZIDE

No	Glucose pesé, g.	Volume de solution, cm.³	Conc. du glucose, %	Semicarbazone recueillie, g.	Glucose trouvé, g.	Moyenne
1	0.1000	10	1.00	0.1835	0.1001	
2	0.1000	10	1.00	0.1840	0.1005	
3	0.1000	10	1.00	0.1826	0.0999	
4	0.1000	10	1.00	0.1830	0.1000	0.10006
5	0.1000	10	1.00	0.1832	0.1001	
6	0.1000	10	1.00	0.1837	0.1003	
7	0.1000	10	1.00	0.1815	0.0993	
8	0.0700	10	0.70	0.1269	0.0693	
9	0.0500	10	0.50	0.0955	0.0522	
10	0.0500	10	0.50	0.0933	0.0512	0.0513
11	0.0500	10	0.50	0.0912	0.0498	
12	0.0500	10	0.50	0.0950	0.0520	
13	0.0200	10	0.20	0.0369	0.0202	
14	0.0200	10	0.20	0.0407	0.0222	0.0208
15	0.0200	10	0.20	0.0373	0.0204	
16	0.0200	10	0.20	0.0376	0.0205	

on évapore jusqu'à volume final de 12 ou 15 cm.³ et on procède comme dans la méthode générale. Voici les résultats qui ont été ainsi obtenus:

TABLEAU II

DOSAGE DU GLUCOSE EN SOLUTION À 0.1% PAR LA *p*-NITROPHÉNYL-4-SEMICARBAZIDE

No	Glucose pesé, g.	Volume de solution, cm.³	Conc. du glucose, %	Semicarbazone recueillie, g.	Glucose trouvé, g.	Moyenne
17	0.0500	50	0.10	0.0950	0.0519	
18	0.0500	50	0.10	0.0952	0.0520	0.0510
19	0.0500	50	0.10	0.0904	0.0494	

Enfin, la méthode a été mise à l'essai, sans défécation préalable, sur des solutions de glucose contenant des substances réductrices, ou sur des urines dont le titre en glucose avait été déterminé au Fehling, en utilisant le ferrocyanure de potassium comme indicateur.

Discussion

La méthode qui vient d'être décrite est essentiellement expérimentale et elle est loin d'être définitive; elle se propose seulement de démontrer la possibilité d'appliquer la réaction bien définie des semicarbazides avec les aldéhydes et les cétones à une détermination quantitative du glucose.

TABLEAU III

DOSAGE DU GLUCOSE EN SOLUTION RÉDUCTRICE PAR LA *p*-NITROPHÉNYL-4-SEMICARBAZIDE

No	Nature de la solution	Pourcentage de glucose	Semicarbazone recueillie, g.	Glucose trouvé, %
20	Solution pure	0.542*	0.0967	0.529
21	Solution pure	0.546*	0.1009	0.552
22	Saturée d'acide urique	0.546*	0.0991	0.542
23	Urine diabétique	0.358**	0.0676	0.370
24	Urine diabétique	1.627**	0.2980	1.656
25	Urine diabétique	0.281**	0.0513	0.272

* *Titre déterminé par pesée du glucose avant dissolution.*
** *Titre déterminé au Fehling avec ferrocyanure.*

Il importe cependant de préciser quelles en sont les limites. On peut d'abord se demander si une solubilité de quelques milligrammes seulement de la *m*-nitrobenzal-*p*-nitrophényl-4-semicarbazone ne pourrait pas avoir, par le jeu des dilutions et des relations arithmétiques du calcul, une influence qui, rapportée au glucose, constitue une erreur appréciable. Le mécanisme même des précipitations empêche cette erreur de se produire: en effet, lors de la première précipitation, celle qui a pour but d'éliminer l'excès de semicarbazide, la *m*-nitrobenzal-semicarbazone a complètement précipité, sauf la petite quantité qui correspond à sa solubilité dans le milieu. Celui-ci se trouve ainsi saturé. La deuxième précipitation fournit une nouvelle quantité de cette semicarbazone, dans un milieu dont les propriétés dissolvantes sont demeurées sensiblement les mêmes; cette nouvelle quantité de semicarbazone précipite donc intégralement, puisque le milieu en était déjà saturé, et son poids correspond avec exactitude à la quantité de glucose-semicarbazone hydrolysée, et par extension, il correspond aussi au poids de glucose présent dans le milieu initial.

La principale limite de la méthode provient de la concurrence qui s'établit entre la fin de précipitation de l'excès de semicarbazide et le commencement de l'hydrolyse de la glucose-semicarbazone; cette hydrolyse a tendance à commencer avant même que la semicarbazide libre n'ait eu le temps de précipiter complètement à l'état de semicarbazone insoluble. De telle sorte que le temps le plus favorable pour cette précipitation (une heure et quarante minutes) correspond probablement au moment où il y a superposition des deux phénomènes avec compensation mutuelle.

Si on contrôle avec soin la durée de cette précipitation, les résultats sont assez précis et uniformes. Mais si on laisse prolonger ce temps au-delà de celui qui a été indiqué, le résultat devient rapidement déficitaire par suite de la disparition d'une partie de la glucose-semicarbazone; ce temps, prolongé à deux heures et demie, conduit à un déficit de 4%. Si au contraire, on abrège le temps offert à la précipitation, le résultat en glucose devient trop fort par

suite de l'élimination incomplète de l'excès de semicarbazide; au cours de l'hydrolyse subséquente de la glucose-semicarbazone, ce résidu de semicarbazide s'ajoute à celle qui provient de l'hydrolyse et conduit ainsi à un résultat trop élevé. Un temps de précipitation de 60 min. seulement a donné un résultat en glucose excédant de 4% le résultat théorique.

La température peut avoir aussi une influence sur la valeur du résultat si elle subit des variations considérables; mais les variations ordinaires de la température du laboratoire (22° à 25°) n'ont pas une influence appréciable.

Bibliographie

1. ARDAGH, E. G. R. et RUTHERFORD, F. C. J. Am. Chem. Soc. 57(6) : 1085-1088. 1935.
2. BARRÉ, R. et PICHÉ, L. Can. J. Research, B, 20(2) : 17-20. 1942.

EFFECT OF INITIAL DRYING TEMPERATURE ON THE APPARENT LIGNIN CONTENT OF PLANT TISSUES[1]

By D. MacDougall[2] and W. A. DeLong[3]

Abstract

A study of the effect of initial drying temperature on the apparent lignin content of various succulent plant tissues has been made. Air-drying, drying *in vacuo* at 60° C., air-drying after removal of constituents soluble in cold water and in cold dilute ethanol, and oven-drying at 105° C. have been compared.

Drying of immature or succulent tissues at 105° C. results in a markedly higher apparent lignin content (as determined by the 72% sulphuric acid method) than is obtained after drying under the other conditions specified. This difference may be of especial importance when it is desired to compare the lignin contents of tissues of different degrees of maturity, or which have received different treatments, as in comparison of ensiled with non-ensiled materials.

The nitrogen and methoxyl contents of the lignin preparations isolated indicate that drying at 105° C. results in the inclusion of nitrogenous or carbohydrate materials, or both, in the lignin fraction. Evidence that the interfering substances are mainly easily soluble and removable prior to desiccation was obtained. It is indicated that non-lignin methoxyl may be present in lignin preparations prepared from tissues dried at 105° C.

At present there is no general agreement among plant chemists as to the temperature at which succulent tissues should be dried prior to the determination of their lignin content. The official A.O.A.C. method (1, p. 143) involves preliminary drying of the tissue at 105° C. Various investigators (9, 10, 11, 12) have employed lower temperatures. A review of the literature indicates, however, that lignin determinations are most frequently made on tissues that have been dried at 100° to 105° C. in air. Nevertheless, there is considerable evidence that suggests that drying at such an elevated temperature is likely to be productive of error. Thus Campbell and Booth (2, 3) found that oven-drying resulted in an increase in the apparent lignin content of both soft and hard woods. At the same time there was a corresponding decrease in furfuraldehyde-yielding material suggestive of condensation of the latter with lignin.

Waksman and Iyer (15) report experiments in which protein was added to lignin preparations. They maintain that formation of a ligno-protein took place and that the resistance of such a complex to microbial decomposition was rendered greater by drying and by an elevated temperature of formation. It seems probable that similar complexes may be formed in succulent tissues on drying at an elevated temperature, or, if such are previously formed, they may at least be rendered more resistant to subsequent decomposition.

[1] *Manuscript received November 28, 1941.*

Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Que. From part of a thesis by D. MacDougall in partial fulfilment of the requirements for the degree of Master of Science. Macdonald College Journal Series No. 169.

[2] *Holder of a bursary under the National Research Council of Canada 1940-41.*

[3] *Assistant Professor of Chemistry.*

Wagner (14) carried out drying experiments on leaves of fodder beets, sweet fodder beets, and sugar beets. He dried samples of these tissues at 60° C. and at 105° to 110° C. and found that the digestibility of the protein was considerably reduced; this was particularly true in regard to the material dried at the higher temperature. It is possible that this effect was due to condensation of protein with lignin to form an indigestible complex.

With the object of obtaining further information on the effect of the conditions of the drying of plant tissues on their apparent lignin content the following materials have been analyzed; Swede turnips (roots), young winter rye (leaves), silage prepared from young winter rye, rhubarb (petioles), and garden beets (roots). The preparation of these materials for analysis is described below.

A. Preparation of Material

Turnip

Only the central part of turnip roots (free from brown heart) was used. The portion of the tissue outside the xylem was removed. The tissues thus selected were ground in a food chopper through a plate with holes of 9 mm. diameter. The ground tissue from several turnips was mixed, and 500-gm. portions were weighed out for drying. Samples I and II were dried at 105° C. in an electric air-oven; I*a* and II*a* were dried in the vacuum oven at 60° at an average pressure of 25 to 28 in. of mercury. The dried tissue was ground in a Wiley mill to pass a screen with circular openings of 0.5 mm. diameter.

Turnip samples Nos. I and I*a* were taken from freshly harvested roots, whereas II and II*a* were from roots that had been kept in cellar-storage for approximately six weeks.

Winter Rye

The rye was sown in the early part of August and harvested at the end of October.

Representative samples of the young plants were taken for analysis at the time the crop was ensiled. These were prepared in the same way as turnip samples Nos. I and I*a*, i.e., (i) oven-dried, and (ii) vacuum-dried at 60° C. The samples to be oven-dried were in this case placed in wire-bottomed trays and then put into a large oven where a current of hot air was drawn up through them. The temperature of drying was 103° C.

Winter Rye Silage

The silos were filled about the end of October, 1940, and the samples for analysis were taken during the last week of March, 1941. Silo I (Samples I, I*a*) was filled with rye plants only. In the case of Silo II, phosphoric acid was added to the rye plants in sufficient quantity to give a 4% concentration. Samples from each of these silos were dried both at 103° C. in the hot air oven (I, II) and at room temperature by being spread out on the desk top (I*b*, II*b*). All samples were then ground in a Wiley mill to pass a 0.5 mm. screen. The oven-dried material was redried at 105° C. and portions of the air-dry samples were also dried at this temperature to determine their residual

moisture content. The residue remaining after pretreatment* of the oven-dried material was redried at 105° C., while that from the air-dried material was dried *in vacuo* at 60° C., before the final lignin determinations were carried out.

Rhubarb

The rhubarb was gathered early in the morning. Only stalks of fairly uniform age were used. The leaves and the bases of the petioles were removed and the stalks were carefully washed to remove all sand and other adhering material. The excess moisture from washing was allowed to dry off at room temperature. The stalks were arranged according to size and the same number of stalks from the various size groups were put into each of three samples. These were then weighed.

Sample I was cut into transverse sections of about one-quarter inch in length and dried to constant weight in the air-oven at 105° C. The dried tissue was then ground in a Wiley mill to pass the 0.5 mm. sieve.

Samples II and II*a* were cut into small pieces and shredded with water in a Model B "Waring Blender"†. For each batch of cut tissue 200 ml. of water was first put into the shredder and rhubarb added in very thin slices until the mixture was too viscous to allow efficient shredding.

The material was then transferred to 250-ml. centrifuge bottles for separation of the solid phase. It was found that adding 25 ml. of 95% alcohol to the mixture in each bottle so as to give approximately a 10% alcohol mixture facilitated the settling out of the solid material. The mixture was centrifuged for 10 min. at an approximate speed of 2500 r.p.m., and the supernatant liquid was then siphoned off. The residues were combined and recentrifuged. Water was used to rinse out the centrifuge bottles when combining the residues. The procedure was repeated until the sample was reduced to the smallest possible volume. Approximately 1500 ml. of water was used for the washing and transferring of a kilogram of fresh tissue. This extraction with water and 10% alcohol was carried out at room temperature.

The residual material of both Samples II and II*a* was next covered with 95% alcohol, well stirred, and centrifuged again. The supernatant alcohol was then siphoned off. Sample II was transferred to a large evaporating dish by means of a little more 95% alcohol. The latter was removed by heating on the steam bath, and drying was completed in the air-oven at 105° C. Sample II*a* was transferred to a large Büchner funnel with 95% alcohol and dried by suction at the pump. It was then placed in a small rotary air-drier. This was composed of a cylindrical can on a steel shaft. A pulley was fixed on one end of the shaft so that the can could be turned by means of an electric motor. The ends of the can were removed and replaced by 60-mesh wire gauze. The sample was placed in the can and a current of air was blown through it by means of an electric fan placed at one end.

* *Pretreatment for removal of substances interfering in the lignin determination; see Section B—Methods of Analysis—for details.*

† *Manufactured by Waring Corporation, New York City.*

After six hours in the drier the sample was removed and broken up into very fine pieces and returned to the apparatus for completion of the drying process, which took about eighteen hours. Finally, the sample was finely ground and a portion was dried in the oven at 105° C. to determine its residual moisture content. After pretreatment these samples were again dried in the air-drier prior to determination of their lignin content.

Beets

Fresh beets were gathered on the mornings of July 3 and July 18. They were washed with water and the tops and long tapering ends of the tap roots were removed. On each date the beets were arranged according to size and divided into two portions. The average weight per beet of the four samples was: I—39.2 gm., I*a*—41.1 gm., II—88.5 gm., and II*a*—87.2 gm. Samples I and I*a* were collected on July 3, II and II*a* on July 18.

Samples (I, II) were weighed, sliced into large evaporating dishes, and placed in the air-oven at 105° C. to dry. Samples I*a* and II*a* were shredded in the "Waring Blender" in the same way as the rhubarb. In this case it was found necessary to add alcohol for the second as well as for the first centrifuging. Each sample was concentrated into four 250 ml. centrifuge bottles and then transferred to a large Büchner funnel. Approximately three litres of water were used for the shredding and transferring of one kilogram of fresh material. The residues were next washed with 95% alcohol until the filtrates were nearly colourless. After removing as much alcohol as possible at the pump, the material was transferred to the rotary drier for completion of the drying process at room temperature. After pretreatment these samples were redried in the air-drier prior to lignin determination. The moisture content of the air-dried pretreated beet and rhubarb tissues was low, being of the order of 8%.

B. Methods of Analysis

Lignin Determinations

It was decided to determine the lignin content of the various tissues by the 72% sulphuric acid method, as this procedure has been most widely used by earlier workers. All the tissues were pretreated by extracting with alcohol-benzene, hot water, and 1% hydrochloric acid solution as prescribed in the official A.O.A.C. method (1, p. 143).

The extracted and dried samples were ground in an iron mortar to pass a 40-mesh sieve. The ground material was dried to constant weight at the appropriate temperature (60°, 105° C., etc.) and the actual lignin determinations were carried out exactly as described by Manning and DeLong (6). It was found that if the sample was allowed to stand for one-half to one hour after refluxing with 3% sulphuric acid, filtration was facilitated. The residues were filtered on asbestos in ignited Gooch crucibles.

To aid filtration, diatomaceous earth was added to the acid mixture and a layer was also placed over the asbestos in the crucible. The filter-aid was ignited for half an hour in the muffle furnace before being used. The residual

lignin was thoroughly washed with hot water and the Gooch crucibles were dried at 105° C., weighed, ignited in the muffle furnace at a dull red heat for half an hour, and reweighed. The loss in weight on ignition was taken as the weight of ash-free lignin obtained from the original sample.

Nitrogen Determinations

Samples for nitrogen determinations were filtered in the same way as described above except that the ignited crucibles and diatomaceous earth were dried at 105° C. both before and after filtering. The increase in weight represented the amount of crude lignin in the original material.

The percentages of nitrogen and methoxyl are both calculated on crude lignin. It was not possible to make a correction for ash content, as the amount of ash found in various lignin preparations from the same material was somewhat variable. However, the ash content of the lignin isolated from turnip, rhubarb, and beet samples was almost negligible, and thus there is very little error from this source in the nitrogen and methoxyl values for these substances. On the other hand, lignin isolated from fresh rye and rye silage contained from 5 to 10% ash, and therefore the nitrogen and methoxyl values found for these materials are somewhat low.

In washing the residues for nitrogen determinations care was taken to leave no lignin particles adhering to the inner walls of the crucibles, from which they could be removed only with difficulty when dry. The entire contents of the crucibles was transferred to 500-ml. Kjeldahl flasks and rinsed down with distilled water. The digestion mixture consisted of 4 gm. of potassium sulphate, 1 gm. of mercuric acetate, and 15 ml. of concentrated c.p. sulphuric acid. After digesting to a small volume, the inner walls of the flasks were washed down with distilled water, a 5 ml. portion of concentrated sulphuric acid was added, and digestion was continued for half an hour after the mixture had become colourless. The digest was cooled, filtered into a 100 ml. volumetric flask, and diluted to volume. Ten-ml. portions of this solution were used in the micro-distillation apparatus. The distillation was carried out as described by Pregl (13).

Methoxyl Determinations

Samples for methoxyl determinations were filtered on sintered glass crucibles of porosity G 2 and G 3. Naphthalene was used to speed filtering as suggested by Mueller and Herrmann (7). After filtration the naphthalene was sublimed on the hot water-bath and the residual lignin was then dried in the usual manner and weighed. The lignin filtered in this way was very easily removed from the crucibles, and any possible contamination of the sample due to particles of glass scraped from the crucible in removing the lignin was avoided. The methoxyl determinations were carried out according to the Vieboch and Schwappach modification of the Ziesel method as described by Clark (4). The hydriodic acid used in these determinations was prepared according to the method described by Clark (5).

Results and Discussion

The lignin values in Table I are expressed as percentage of ash-free lignin in oven-dry material. In the case of the rhubarb and beet samples, which were extracted before drying, the percentage dry weight was calculated from the dry weight of the samples dried at 105° C. Portions of the material dried at low temperatures were subsequently dried at 105° C., so that their lignin contents could be calculated on an oven–dry-weight basis.

TABLE I

THE EFFECT OF TEMPERATURE OF PRELIMINARY DRYING ON THE APPARENT LIGNIN CONTENT OF VARIOUS PLANT TISSUES

Material	Sample	Loss in dry weight in pretreatment, %	Ash-free lignin, dry weight basis, %	Nitrogen in crude lignin, %	Methoxyl in crude lignin, %	Remarks
Turnip	I*	84.2	6.27 ± 0.07 (3)‡	6.68	0.406	Newly harvested roots
	I_a†	90.0	1.31 ± 0.02 (3)			
	II	88.7	4.84 ± 0.09 (3)	4.26	0.968	Roots kept in cellar-storage for about six weeks
	II_a	92.6	1.13 ± 0.04 (3)	8.50	1.43	
Young rye	I	70.6	6.08 ± 0.23 (3)	6.46	1.69	Fresh material
	I_a	76.8	4.28 ± 0.01 (3)	4.79	2.21	
Silage	I	67.3	8.86 ± 0.04 (3)	6.98	1.61	Silage made from young rye
	I_b	73.0	4.06 ± 0.08 (3)	4.21	1.53	
	II	70.7	7.86 ± 0.09 (6)	5.57	1.51	Rye silage + 4% phosphoric acid
	II_b	73.0	4.20 ± 0.07 (6)	3.46	1.83	
Rhubarb	I	70.1	13.6 ± 0.2 (3)	3.95	0.705	Fresh rhubarb
	II	82.4	2.74 ± 0.02 (3)	5.36	2.88	Fresh rhubarb from which soluble materials were washed before drying
	II_c	82.5	1.73 ± 0.03 (3)	3.65	5.53	
Beets	I	86.6	2.82 ± 0.15 (3)	8.16	1.13	Beets harvested July 3
	I_c	93.5	0.550 ± 0.014 (3)	5.48	3.14	
	II	84.2	6.34 ± 0.25 (3)	5.84	0.541	Beets harvested July 18
	II_c	92.9	0.554 ± 0.02 (3)	6.79	2.61	

* *Sample numbers without subscript letters indicate that the samples were dried at 100° to 105° C.*

† *Samples marked (a) were dried in vacuo at 60° C.; those marked (b) were air-dried at room temperature; and those marked (c) were air-dried at room temperature after their easily soluble materials had been washed out with cold water and dilute alcohol.*

‡ *The percentage of lignin is followed by the average deviation and, in parentheses, the number of determinations carried out.*

The data of Table I show clearly that the temperature of preliminary drying has a marked effect on the apparent lignin content of the tissues analysed. In every case the apparent lignin content of samples dried at a low temperature is much lower than that of samples of the same tissue dried at an elevated temperature. This result may probably be ascribed to formation of artifacts

and to a greater condensation of nitrogenous and carbohydrate materials with the lignin at the higher temperatures. It is also possible that drying at high temperatures caused a change in the molecular constitution of the lignin itself and rendered it more insoluble in the reagents used for the pretreatments and lignin determinations. Of these two possibilities the former seems the more probable and has considerable experimental evidence in its favour (8, p. 153). However, it may be that a part of the fraction which some workers have called "soluble" lignin is being determined in the oven-dried material but not in that dried at room temperature. Evidence in support of such a hypothesis is difficult to obtain, since accurate determination of small amounts of "soluble" lignin in the pretreatment extracts, accompanied as they are by considerable quantities of carbohydrates and nitrogenous materials, is not practicable at present.

In any event, the nitrogen and methoxyl contents of the isolated lignin would seem to indicate that the interference avoided by low temperature drying is much greater than any error likely to be caused by solution of lignin.

In every tissue studied, the absolute amount of nitrogen in the residue from material dried at elevated temperatures was much greater than from tissue dried at low temperatures. In some instances there was 8 or 10 times as much nitrogen in the residue from the oven-dried sample as in that from the material dried at low temperature. In no case was the absolute amount of methoxyl as great in the "low temperature" lignin as in the other, although the percentage was usually higher in the former. This may be due to condensation of methoxyl-containing carbohydrates, probably of a hemicellulosic nature, with the lignin on drying at elevated temperatures. That the lignin from material dried at a low temperature is purer than that from material dried at elevated temperatures is indicated by the fact that the absolute amount of nitrogen in the former was always much lower and the percentage of methoxyl usually higher. As indicated above, however, the nitrogen content is probably the safer criterion of purity.

Comparison of the results for rhubarb samples Nos. I and II would seem to indicate that it is the easily soluble plant constituents that cause the bulk of the interference in material dried at high temperatures. The only difference between the treatments given to Samples I and II was that the latter had its easily soluble constituents washed out with water and approximately 10% alcohol prior to drying at 105° C. Yet, Sample I had almost five times as much apparent lignin as Sample II. The interference in Sample I is, perhaps, largely due to formation of humin-like substances from easily soluble carbohydrate and nitrogenous materials. On the other hand, comparison of rhubarb samples Nos. II and II*c* shows that the whole interference is not caused by readily soluble materials. Both samples had their readily soluble constituents removed prior to drying, then Sample II was dried at 105° C. and Sample II*c* at room temperature. Sample II showed an apparent lignin content of 2.74% while Sample II*c* had only 1.73% lignin. These results indicate that the main interference in the determination of the lignin content

of succulent tissues dried at high temperatures is due to formation of humin-like substances from readily soluble plant constituents. There is also a smaller but still significant interference due to water insoluble material.

The hypothesis suggested to explain the results obtained in the analysis of the rhubarb samples is supported by a comparison of the percentages of dry weight lost by the samples dried at high and at low temperatures in the pretreatment extractions with alcohol-benzene, hot water, and 1% hydrochloric acid. Such a comparison shows that in every instance more material was removed from the tissue dried at low temperature. However, the differences in amount lost by pretreatment are often slight and never proportional to the lowering of the apparent lignin content of the material. It would seem that high temperature drying renders a part of the readily soluble plant constituents insoluble in both the pretreatment solvents and in the concentrated acid, especially the latter. On the other hand, although the readily soluble constituents of the samples dried at low temperature appear to be rendered virtually insoluble in alcohol-benzene, hot water, and dilute hydrochloric acid, they, as well as more difficultly soluble substances, can still be removed by the concentrated acid treatment or by the hydrolysis with dilute acid which follows it.

The fact that the apparent lignin content of material dried at low temperatures shows very little variation with changes in the nature of the other plant constituents would seem to indicate that probably a relatively pure substance is being isolated. Changes in the composition of beet roots with increasing age and of Swede turnip roots on storing have a marked effect on the amount of lignin residue obtained from oven-dried samples of these tissues, but have little or no effect on the amount of lignin obtained from samples dried at a low temperature.

A comparison of the results for fresh rye and rye silage when both were dried at 100° to 105° C. indicates that ensiling causes a marked increase in lignin as percentage of dry weight. However, during the ensiling period there was a loss of approximately 16.8% of the original dry weight from both silos. This loss is presumably due almost entirely to decomposition of readily soluble carbohydrates. If it is assumed that there was no loss of lignin during the ensiling period, then, on the basis of the values obtained for fresh material, we would expect to obtain 7.31% lignin in the silage. The actual values obtained were significantly higher than the expected, viz., 8.86 and 7.86%, and it would thus appear that high temperature drying caused an even greater error with the silage than with the fresh material.

If samples are dried at low temperatures the apparent effect of ensiling seems to be to lower the lignin content of young rye. Based on the results for fresh material, the silage should contain approximately 5.14% lignin when dried at low temperature. The actual results, 4.06 and 4.20%, were much lower than the expected. This discrepancy may be due to the fact that the fresh material was vacuum-dried at 60° C., whereas the silage samples were air-dried at room temperature before the lignin determinations were

carried out. Thus the results on these materials cannot be compared and it would appear that drying at 60° C. results in the inclusion of some non-lignin material with the lignin fraction. Further investigation of this point is necessary.

References

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis. 5th ed. A.O.A.C., Washington, D.C. 1940.
2. Campbell, W. G. and Booth, J. Biochem. J. 24 (3) : 641-648. 1930.
3. Campbell, W. G. and Booth, J. Biochem. J. 25 (3) : 756-762. 1931.
4. Clark, E. P. J. Assoc. Official Agr. Chem. 15 (1) : 136-140. 1932.
5. Clark, E. P. Ind. Eng. Chem Anal. Ed. 10 (12) : 677. 1938.
6. Manning, K. R. and DeLong, W. A. Sci. Agr. 22 (2) : 69-79. 1941.
7. Mueller, W. J. and Herrmann, W. Tech.-Wiss. Teil, Papierfabr. 24 : 185. 1926. *Abstracted in* Chem. Abstracts, 20 (16) : 2746. 1926.
8. Norman, A. G. The biochemistry of cellulose, the polyuronides, lignin, etc. Oxford University Press, New York and London. 1937.
9. Peterson, C. J. and Hixon, R. M. Ind. Eng. Chem. Anal. Ed. 1 (2) : 65-67. 1929.
10. Peterson, C. J., Walde, A. W., and Hixon, R. M. Ind. Eng. Chem. Anal. Ed. 4 (2) : 216-217. 1932.
11. Phillips, M., Goss, M. J., Davis, B. L., and Stevens, H. J Agr. Research, 59 (5) : 319-366. 1939.
12. Platenius, H. Plant Physiol. 9 (3) : 671-680. 1934.
13. Pregl, F. Quantitative organic microanalysis. 3rd English ed. J. & A. Churchill, Ltd., London. 1937.
14. Wagner, H. Landw. Z. 40 : 441; Z. Ver. deut. Zuckerind. 71 : 251. 1921. *Abstracted in* Chem. Abstracts, 15 (15) : 2519. 1921.
15. Waksman, S. A. and Iyer, K. R. N. Soil Sci. 36 (1) : 69-82. 1933.

THE ALKALOIDS OF FUMARIACEOUS PLANTS

XXXI. *CORYDALIS MONTANA* (ENGELM.) BRITTON[1]

By Richard H. F. Manske[2]

Abstract

Seven known alkaloids have been isolated from *Corydalis montana* (Engelm.) Britton, namely: protopine, *dl*-tetrahydro-palmatine, corydaline, capauridine, capaurine, scoulerine, and capaurimine. Two new bases have been isolated. One of these, alkaloid F57, was obtained by reduction of a quaternary base which itself could not be obtained crystalline. *C. aurea* has also yielded the same base. It is represented by $C_{18}H_{21}O_3N$ containing three methoxyls. The second new base, alkaloid F56, is represented by $C_{23}H_{27}O_6N$ containing four methoxyl groups.

It is pointed out that the chemical differences between *C. aurea* and *C. montana* are of sufficient magnitude to allocate specific rank to each plant.

Corydalis montana (Engelm.) Britton is so closely related to *C. aurea* Willd. that it was classed as variety *occidentalis* of the latter by Engelmann. The habits of the plants and the morphology of their flowers are so similar that only critical examination by a competent botanist can serve to authenticate a series of specimens. The writer is therefore particularly grateful to Dr. H. A. Senn, Botanist, Department of Agriculture, Ottawa, who, in collaboration with Dr. G. H. M. Lawrence of the Bailey Hortorium, Cornell University, Ithaca, N.Y., undertook such an examination. They consider it a good species distinct from *C. aurea* Willd. Specimens are deposited in the herbarium of the Bailey Hortorium and in the herbarium of the Division of Botany and Plant Pathology, Department of Agriculture, Ottawa.

Because of lack of material it was not possible to subject *C. montana* to as thorough an examination as in the case of *C. aurea*. It was nevertheless possible to isolate a total of nine alkaloids, six of which, namely, protopine, *dl*-tetrahydro-palmatine, capaurine, capauridine, corydaline, and a new base, F57, are now known to occur in *C. aurea* (1, 3). Two of the remaining three, namely, capaurimine and scoulerine, had previously been isolated from *C. pallida* (4) and *C. scouleri* (2) respectively. The last, alkaloid F56, was obtained only in very small amounts so that it could not be characterized apart from its analyses, which yielded figures in good agreement with $C_{23}H_{27}O_6N$ containing four methoxyl groups.

It is pertinent to point out that the chemical examination had been completed before the plant had been properly identified, although it had been tentatively regarded as *C. montana*. On chemical grounds alone it now seems reasonable to allocate to it specific rather than varietal rank.

[1] *Manuscript received February 2, 1942.*

Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada. Issued as N.R.C. No. 1044.

[2] *Chemist.*

Experimental

The material for the present investigation was grown in a local garden from seed which came from a single plant. When dried and ground it weighed 9.0 kilos.

The following is a summary of the various fractions and the contained alkaloids.

Base hydrochlorides extracted from aqueous solution by means of chloroform,

BC—Non-phenolic bases,—*dl*-tetrahydro-palmatine, corydaline.
EC—Phenolic bases extracted from alkaline solution by means of ether,—alkaloid F56.
BCE + EEC—Phenolic bases precipitated by carbon dioxide,—capaurine, capauridine.
CEC—Phenolic bases not precipitated by carbon dioxide but extracted by means of chloroform,—alkaloid F57.

Base hydrochlorides not extracted from aqueous solution by means of chloroform,

BS—Non-phenolic bases,—protopine.
BSE+EES—Phenolic bases precipitated by carbon dioxide,—scoulerine, capaurimine.

dl-Tetrahydro-palmatine and Corydaline

The main fraction of the alkaloid separated as the sparingly soluble hydrochloride when the acidic solution was extracted with ether prior to basification. The free base was regenerated and recrystallized from methanol. It then melted at 150° C.*, either alone or in admixture with an authentic specimen of *dl*-tetrahydro-palmatine. The yield was 0.04%.

The bases from the more soluble hydrochlorides were extracted from the basified solution with ether. The residue in contact with methanol yielded a small amount of protopine. It was therefore redissolved in dilute hydrochloric acid and the solution extracted with chloroform to eliminate the BS bases. The residue from the chloroform extract was redissolved in water, the filtered solution basified with ammonia, and the bases were extracted with ether. The residue from the extract crystallized readily in contact with methanol. The base was recrystallized from hot methanol, and it then consisted of the characteristic stout tablets of corydaline melting at 136° C. either alone or in admixture with an authentic specimen. Yield, 0.01%.

Alkaloid F56

The fraction EC deposited a very sparingly soluble base which, after washing with methanol, melted indefinitely at 196° C. It was recrystallized twice from chloroform-methanol, and it then melted at 207° C. In admixture with capauridine, which it resembles in appearance and which occurs in this fraction, it was completely liquid at 190° C. Its solution in sulphuric acid is pale olive in colour which merely changes to brown on heating. The analytical figures agree best with $C_{23}H_{27}O_6N$ containing four methoxyl groups. Calcd. for $C_{23}H_{27}O_6N$: C, 66.83; H, 6.54; N, 3.39; 4 OMe, 30.04%. Found: C, 67.12, 66.94; H, 6.49, 6.70; N, 3.44, 3.12; OMe, 29.96%.

* *All melting points are corrected.*

Capauridine and Capaurine

The fractions BCE and EEC in contact with methanol deposited a small amount of base which was recrystallized from chloroform-methanol. It melted at 207° C. and admixture with a specimen of capauridine did not lower the melting point. Yield, 0.003%.

The bases from the mother liquor from the capauridine as well as the totally phenolic fraction of EC were dissolved in dilute acid, the bases regenerated from the filtered solution, and extracted with ether. The extract was evaporated to a small volume and treated with just sufficient methanol to redissolve the resin that had separated. The base which then crystallized was recrystallized first from methanol-ether and then from methanol. It was obtained in stout prisms that melted at 164° C. either alone or in admixture with capaurine. Yield, 0.003%.

Alkaloid F57

The aqueous carbonated solution from which ether extraction has removed fraction EEC is in general free of organic bases. In the case of some plants, however, extraction with chloroform removes a not inappreciable amount of base. *C. aurea* and the plant under consideration yielded a comparatively large amount of a water soluble uncrystallizable resin. The dark golden colour suggested that a substantial amount of protoberberine bases might be present. The residues from the two different plants were therefore subjected to reduction with zinc dust in dilute acetic acid in parallel experiments until the colour had faded to a pale yellow. The cooled and filtered solutions were basified with ammonia and extracted with ether. The residues in contact with methanol crystallized readily and yielded the same base melting at 127° C. It was recrystallized from hot methanol in which it is only moderately soluble. The colourless prisms thus obtained melted sharply at 129° C. and proved to be non-phenolic. This base, alkaloid F57, is best represented by $C_{18}H_{21}O_3N$ containing three methoxyl groups. Calcd. for $C_{18}H_{21}O_3N$: C, 72.24; H, 7.02; N, 4.68; 3 OMe, 31.10%; mol. wt. 299. Found: C, 71.58, 71.82; H, 7.31, 7.38; N, 4.51, 4.24; OMe, 32.48%; mol. wt. 338.

Protopine

The fraction BS yielded no base other than protopine, which was readily obtained in a state of high purity; m.p. 210° C. The yield was 0.07%.

Scoulerine and Capaurimine

Neither fraction BSE nor EES deposited a crystalline base in contact with methanol. They were combined, dissolved in dilute hydrochloric acid, the bases regenerated from the filtered solution, and extracted with ether. The residue from the ether solution was neutralized in methanol with anhydrous hydrogen chloride, and the somewhat concentrated solution treated with ethyl acetate until the incipient turbidity just disappeared on mixing. In the course of several days a crystalline hydrochloride separated. The addition of ethyl acetate was continued from day to day until no further crystalline

product was obtained. It was filtered off and washed with methanol–ethyl-acetate. As thus obtained, the hydrochloride consisted of a mixture, one component of which was virtually insoluble in cold water and the other readily soluble. The less soluble hydrochloride when shaken with ammonia and ether yielded several milligrams of a base that melted not quite sharply at 200° C. This melting point was not depressed when the base was mixed with a specimen of scoulerine from *C. scouleri*.

The base regenerated from the more soluble hydrochloride crystallized from ether, and when repeatedly recrystallized from methanol-ether it melted at 213° C. A further small amount of this base was obtained from the methanol–ethyl-acetate, another liquor from which the hydrochlorides had been obtained. This base in admixture with a specimen of capaurimine from *C. pallida* melted at 212° C. A methoxyl determination yielded 27.07%. Three methoxyls in $C_{20}H_{23}O_5N$ require 26.05%.

References

1. MANSKE, R. H. F. Can. J. Research, 9(11) : 436-442. 1933.
2. MANSKE, R. H. F. Can. J. Research, B, 14(10) : 347-353. 1936.
3. MANSKE, R. H. F. Can. J. Research, B, 16(3) : 81-90. 1938.
4. MANSKE, R. H. F. Can. J. Research, B, 18(3) : 80-83. 1940.

CANADIAN JOURNAL OF RESEARCH

Notes on the Preparation of Copy

General:—Manuscripts should be typewritten, double spaced, and the *original* and *one copy* submitted. Style, arrangement, spelling, and abbreviations should conform to the usage of this Journal. Names of all simple compounds, rather than their formulae, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed. Manuscripts should be carefully checked before being submitted, to reduce the need for changes after the type has been set. All pages should be numbered.

Abstract:—An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required.

Illustrations

(i) *Line Drawings:*—Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in *blue* only. Paper ruled in green, yellow, or red should not be used. The principal co-ordinate lines should be ruled in India ink and all lines should be of sufficient thickness to reproduce well. Lettering and numerals should be of such size that they will not be less than one millimetre in height when reproduced in a cut three inches wide. If means for neat lettering are not available, lettering should be indicated in pencil only. All experimental points should be carefully drawn with instruments. Illustrations need not be more than two or three times the size of the desired reproduction, but the ratio of height to width should conform with that of the type page. The *original drawings* and one set of small but clear *photographic copies* are to be submitted.

(ii) *Photographs:*—Prints should be made on glossy paper, with strong contrasts; they should be trimmed to remove all extraneous material so that essential features only are shown. Photographs should be submitted *in duplicate;* if they are to be reproduced in groups, one set should be so arranged and mounted on cardboard with rubber cement; the duplicate set should be unmounted.

(iii) *General:*—The author's name, title of paper, and figure number should be written on the back of each illustration. Captions should not be written on the illustrations, but typed on a separate page of the manuscript. All figures (including each figure of the plates) should be numbered consecutively from 1 up (arabic numerals). Reference to each figure should be made in the text.

Tables:—Titles should be given for all tables, which should be numbered in Roman numerals. Column heads should be brief and textual matter in tables confined to a minimum. Reference to each table should be made in the text.

References should be listed alphabetically by authors' names, numbered in that order, and placed at the end of the paper. The form of literature citation should be that used in this Journal and titles of papers should not be given. All citations should be checked with the original articles. Each citation should be referred to in the text by means of the key number.

The *Canadian Journal of Research* conforms in general with the practice outlined in the *Canadian Government Editorial Style Manual*, published by the Department of Public Printing and Stationery, Ottawa.